Serum glutathione S-transferase B_1 activity as an index of liver function in cystic fibrosis

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Abstract

***Aims*—To evaluate serum glutathione S-transferase B_1 (GST B_1), a highly sensitive test of hepatocellular function, as a means of identifying liver disease in patients with cystic fibrosis (CF).**

***Methods*—The presence of liver disease was sought over a three year period in 60 children with CF, using a combination of clinical assessment, ultrasound examination, conventional biochemical tests of liver function (LFTs), and measurement of GST B_1.**

***Results*—Reference ranges for serum GST B_1 were established in a paediatric control population. The 95% value (4·55 μg/l) was similar to the upper limit of normal previously derived in adults. Mean (SE) serum GST B_1 activities were higher in the CF population (9·0 (1·14) μg/l) than in age matched controls (2·4 (0·15) μg/l). Ten patients with CF showed clinical signs of liver dysfunction. All but one had a serum GST B_1 >4·55 μg/l. Twelve other patients had elevated LFTs without clinically evident liver dysfunction, six had abnormal ultrasound scans and two showed both of these anomalies. Thirty patients with CF had neither biochemical, ultrasonographic nor clinical signs of liver disease. On review three years later, clinically important liver disease was reaffirmed in eight of the 10 index cases and had become apparent in a further eight, all of whom had elevated GST B_1 activities. Five (36%) of the patients with elevated LFTs and two (33%) with isolated ultrasound changes continued to show these abnormalities.**

***Conclusions*—The limitations of conventional LFTs and ultrasound scans were evident from this study. The results suggest that elevated GST B_1 activities may be a better predictor of hepatic dysfunction in CF than conventional LFTs.**

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Keywords: Glutathione S-transferase B_1 cystic fibrosis, liver disease, liver function tests, ultrasound.

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Liver involvement occurs in 20–50% of patients with cystic fibrosis, depending on age and the type of pathology recognised.[1 2] Steatosis, which is independent of age, occurs in one third of patients[1] but may regress with improved nutrition. Focal biliary cirrhosis, which is pathognomonic of cystic fibrosis,[3] shows an increasing incidence with age, rising from 10% in children dying within the first three months to 27% in children dying after the first year.[4] Perhaps 5% of children with focal biliary cirrhosis will go on to develop multinodular cirrhosis.

The prevalence of clinically apparent liver disease also shows a progressive increase with age to a peak of 8·7% in 16–20 year olds[2] and appears to be unrelated to the severity of disease elsewhere.[5] Hepatobiliary disease is, however, frequently asymptomatic, with overt liver disease recognised in <5% of patients with cystic fibrosis.[2] Biochemical abnormalities are more prevalent (12·9%) but correlate poorly with clinical findings[6] and often remain unremarkable until liver dysfunction becomes clinically evident.[7] A non-invasive test to detect hepatic dysfunction in its early stages and monitor progression would therefore be a desirable adjunct to patient management, particularly as recent studies suggest that therapy with ursodeoxycholic acid (UDCA) may arrest or reverse the disease process.[8]

The cytosolic glutathione S-transferases (GSTs) are a family of dimeric detoxification enzymes with at least three classes of GSTs demonstrable in humans. The liver contains high concentrations of the α class GSTs which are composed of two immunologically distinct subunits B_1 and B_2. When measured using radioimmunoassay,[9] serum or plasma GST B_1 concentrations can provide a more sensitive index of liver function than aminotransferase activity.[10 11] We have therefore measured serum GST B_1 in control subjects and in patients with cystic fibrosis aged between three weeks and 18 years to investigate the relation of GST B_1 with other indexes of liver disease in cystic fibrosis.

Methods

Sixty four children, 25 girls and 37 boys, mean age 7·7 years, range three weeks to 18 years, attending the cystic fibrosis clinic at the Children's Hospital, Sheffield, were studied. All patients had undergone repeated abdominal examination by one of two paediatricians or a cystic fibrosis research fellow during routine monthly clinic visits. Those with clinical evidence of liver disease were identified.[5] Annual review data were scrutinised to establish age at diagnosis of liver disease, mode of progression, presence of oesophageal varices, and the results of hepatic ultrasound scans and liver function tests. Patients were followed for a three year period with monthly clinic visits, regular abdominal examinations and annual assessments including hepatic ultrasound scans and liver function tests. The presence or absence of

Table 1 Results for children with cystic fibrosis who had clinical evidence of liver disease at initial (1990) and/or later (1993) examination

Bilirubin (mmol/l)	ALP (IU/l)	γGT (IU/l)	AST (IU/l)	ALT (IU/l)	Total protein (g/l)	Albumin (g/l)	GST B_1 (μg/l)	Age/Sex (years)	Clinical liver disease		Ultrasound		Liver histology
									1990	1993	1990	1993	
6	741	40	72	87	76	38	31·9	12·80/M	Yes	Yes	Abnormal	Normal	Not done
5	590	244	118	148	78	51	31·3	1·25/M	Yes	Yes	Abnormal	Abnormal	Cirrhosis
4	692	40	31	35	72	42	25·7	12·20/F	Yes	Yes	Abnormal	Abnormal	Not done
5	681	54	81	62	66	44	25·2	8·17/M	Yes	Yes	Normal	Abnormal	Cirrhosis
4	720	44	89	48	71	44	16·0	11·40/F	Yes	Yes	Normal	Abnormal	Not done
7	727	47	47	41	73	44	14·9	9·41/M	Yes	Yes	Normal	Normal	Cirrhosis
11	1817	260	108	100	74	44	9·2	15·0/M	Yes	Yes	Normal	Abnormal	Cirrhosis
7	508	14	24	17	64	40	5·2	5·00/F	Yes	No	Normal	Normal	Not done
8	548	25	24	21	79	43	4·7	15·50/M	Yes	Yes	Abnormal	Normal	Not done
10	574	17	19	12	69	46	1·9	12·10/F	Yes	No	Normal	Normal	Not done
5	668	20	108	67	68	47	32·0	0·96/M	No	Yes			
4	563	4	47	54	63	42	10·6	1·00/M	No	Yes			
4	562	59	126	12	70	41	7·2	16·08/M	No	Yes			
7	575	30	45	41	75	43	16·0	3·67/F	No	Yes			
4	989	4	28	14	67	46	8·4	1·17/M	No	Yes			
4	248	13	24	7	75	41	9·6	15·20/F	No	Yes			
7	184	13	18	33	56	34	11·3	12·00/M	No	Yes			
5	617	25	124	135	75	43	31·0	12·80/F	No	Yes			
*<20	100–650	<45	<40	<30	65–80	35–45							

* Reference ranges in older children, age related ranges were used to assess abnormality.

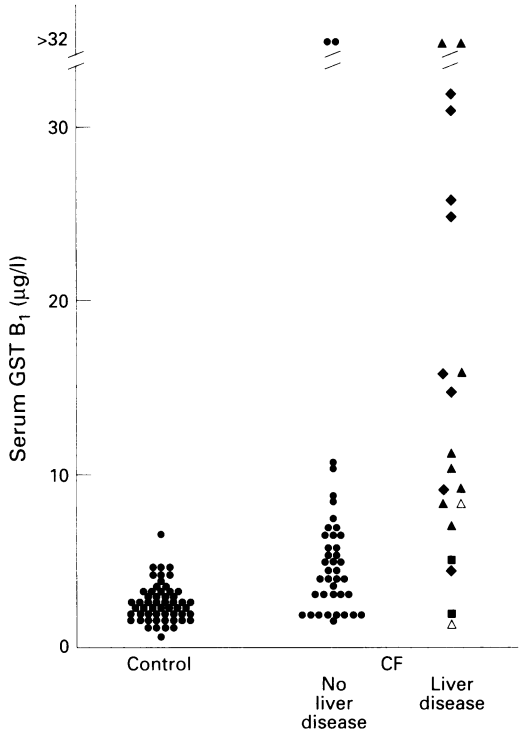

Figure 1 Serum GST B_1 activities for controls and patients with cystic fibrosis (CF) with and without liver disease. ●, Controls and patients with CF without liver disease; ■, patients with CF with clinical liver disease in 1990; ▲, patients with CF with clinical liver disease in 1993; ♦, patients with CF with clinical liver disease in 1990 and 1993; △, patient lost to follow up.

Table 2 Performance characteristics for serum GST B_1 at different normal threshold values when compared with assessments of clinical liver disease in 1990 and 1993

Year of clinical assessment of liver disease	Upper threshold normal value for GST B_1 (μg/l)	Sensitivity	Specificity
1990	30	20·0	92·0
	20	40·0	92·0
	15	50·0	90·0
	10	60·0	82·0
	8	70·0	72·0
	6	70·0	58·0
	5	80·0	46·0
	4	90·0	32·0
	3	90·0	18·0
1993	30	25·0	97·6
	20	37·5	97·6
	15	50·0	97·6
	10	68·8	92·9
	8	87·5	88·1
	6	93·8	73·8
	5	93·8	57·1
	4	100·0	40·5
	3	100·0	23·8

clinical liver disease was reassessed at the end of this period. Four children were excluded from the study; serum samples from two boys were discarded as one had an alkaline phosphatase level of 3000 IU/l with evidence of metabolic bone disease and in the other serum haemolysis precluded analysis; a further child moved away from the area and another died.

Twenty one girls and 34 boys, mean age 8·1 years, with conditions unrelated to cystic fibrosis, and without clinical or biochemical evidence of liver disease, served as controls. Analyses were performed on serum remaining from these children after diagnostic tests had been performed. The study was approved by the Hospital Ethics Committee.

Serum for GST B_1, obtained at onset of the study, was separated from whole blood within one hour of collection and kept at −20°C until analysed. GST B_1 was quantitated by double antibody radioimmunoassay using purified GST B_1 as standard and ^{125}I labelled GST B_1 as tracer. Analytical sensitivity of the assay was enhanced by delayed tracer addition. Analyses were performed in duplicate with samples from age and sex matched patients and controls run as adjacent pairs in seven batches. Unpaired patient and control samples were added throughout the batches. Assay performance was as previously described.[12] Radioimmunoassay results were calculated by an iterative four parameter fit of the lin/log transformed standard curve by RIACALC program (LKB Instruments Ltd, Croydon, UK).

Standard biochemical liver function tests were performed in serum on a Cobas Bio centrifugal analyser (Roche Products Ltd, Welwyn, UK). In-house, age related reference ranges were used to interpret results. Statistical comparisons were by unpaired *t* test or by simple regression except where stated otherwise.

Results

Significant differences existed between cystic fibrosis and control populations for all liver function tests except bilirubin and total protein. There were no significant correlations between serum GST B_1 and age for either group, although a small sex difference was apparent in

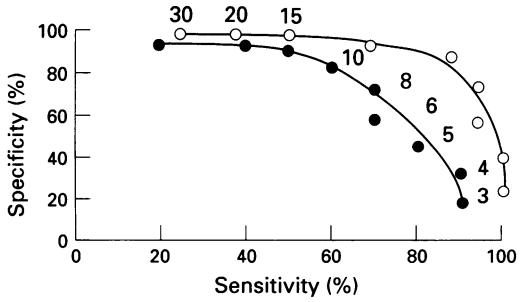

Figure 2 ROC curves for serum GST B_1 at various cut off values when clinical liver disease was assessed in 1990 (●) and 1993 (○).

control serum samples (males $2{\cdot}15 \pm 0{\cdot}9$ μg/l, females $2{\cdot}8 \pm 1{\cdot}4$ μg/l, $p = 0{\cdot}03$); the results for both paired and unpaired subjects were therefore pooled for both groups. Mean (SE) serum GST B_1 activities were significantly higher ($p < 0{\cdot}001$) in patients with cystic fibrosis (9·0 (1·14) μg/l) compared with controls (2·4 (0·15) μg/l). Significant correlations existed in the cystic fibrosis group between GST B_1 and alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (γGT) ($r = 0{\cdot}6$, 0·5 and 0·3, respectively).

The 95% value for serum GST B_1 was calculated for the controls. The derived value (4·55 μg/l) was similar to the upper limit of normal previously established in adult studies (4 μg/l).[11] Four patients with cystic fibrosis gave results above the top serum GST B_1 standard (32 μg/l) but were assigned this value for data analysis.

Ten patients with cystic fibrosis with clinical liver disease (persistent hepatosplenomegaly with or without oesophageal varices) were identified at the onset of the study. All but one had a serum GST B_1 activity >4·55 μg/l (table 1, fig 1). On review three years later, eight of the index cases were still considered clinically abnormal, but one patient with a GST B_1 activity of 5·2 μg/l and the child with a normal GST B_1 activity (1·9 μg/l) no longer exhibited signs of liver disease. During the study period, clinical evidence of liver dysfunction became apparent in a further eight patients, all of whom had elevated GST B_1 activities at the first examination (table 1).

Discussion

The striking feature of hepatic dysfunction in cystic fibrosis is the paucity of clinical signs until the disease is advanced. The poor correlation between cystic fibrosis liver disease and traditional liver function tests may be caused by the focal nature of the hepatic involvement, which predominantly affects biliary tracts.[13] Even when portal tract changes are advanced, parenchymal architecture and liver cell integrity are well preserved, hence portal hypertension and its consequences are prominent whereas hepatocellular failure is rare.[7] Given these limitations, the performance of the GST B_1 assay in detecting cystic fibrosis liver disease, when examined by means of receiver operating characteristic (ROC) curves,[14] was encouraging (table 2).

Serum GST B_1 activities were compared with the results of clinical examinations in both 1990 and 1993 in terms of sensitivity and specificity (table 2). The predictive value of serum GST B_1 for liver disease in cystic fibrosis is shown by an increase in the area under the ROC curves (fig 2). These calculations can also assist the choice of a cut off serum GST B_1 value for the most effective detection of actual or incipient liver disease. For a disease that is serious, treatable, requires detection, and where there are unlikely to be serious consequences for patients with false positive results, then a cut off value with a high sensitivity should be applied. Thus, a cut off value at the upper reference limit for serum GST B_1 (4·55 μg/l) would give a sensitivity of 97% and a specificity of 50% in the present study. This represents the detection of liver disease in all of the patients with cystic fibrosis who manifested disease three years later at the cost of suspecting liver disease in 18 patients who remained asymptomatic. Alternatively, if treatment options for liver disease in cystic fibrosis are considered limited and the main purpose of the test is to reassure patients who are unlikely to develop liver dysfunction, a cut off value at a higher specificity can be selected from the ROC curve (fig 2) or from table 2. A cut off value at 10 μg/l would, for example, have missed five cases of liver disease in the present study but the number of false positive results would have decreased to three. If serum GST B_1 activities predict liver disease in cystic fibrosis over a period greater than three years, it would be expected that the above indexes of test performance should further improve with additional follow up.

The limitations of conventional liver function tests are also evident from this study. Confounding factors include the effect of drug therapy on γGT, and of differential isoenzymes on alkaline phosphatase (ALP). Specific and suitable ALP isoenzymes such as the high molecular mass (biliary) form have, however, been used as a marker of liver disease in cystic fibrosis. With this assay, Schoenau *et al*[15] found similar differences between children with cystic fibrosis and controls to those demonstrated with serum GST B_1 in this study, although no clinical correlations were made. Feigelson *et al*[16] also made a longitudinal study of liver function tests in 50 patients with cystic fibrosis over a three year period. They found that clinical signs of an enlarged hard liver, plus or minus splenomegaly, preceded biochemical changes by several months. Tests of cytolysis (ALT, ornithine carbamyl transferase, and serum B_{12}) in patients with clinical cirrhosis were generally raised with much fluctuation, whilst tests of cholestasis (ALP, leucine aminopeptidase and γGT) were always abnormal. Our initial data were at variance with those of Feigelson *et al* as only 40% of our children with clinical cirrhosis had abnormal γGT activities and 60% had raised ALP activities (table 1). Follow up data, however, improve this comparison as all but one child with confirmed cirrhosis had a raised ALP and four of seven had elevated γGT activities.

Abdominal ultrasound has also been used as a tool to predict liver damage in cystic fibrosis. Hepatic echogenicity is assessed in relation to the right renal cortex and is considered abnormal if an increased or diffuse patchiness is seen.[17] Using this technique, Graham *et al*[18] found no correlation with liver function and concluded that ultrasound was useful in showing organ morphology but not in assessing disease severity. A correlation has been found between ALP activities and portal vein diameters measured ultrasonically in 21 adults with cystic fibrosis.[19] However, the low sensitivity for these measurements in detecting portal hypertension was subsequently demonstrated in a large study of 61 adults with cystic fibrosis liver disease who underwent liver biopsy within three weeks of their liver ultrasound scans.[20] Gosink *et al*[21] found a false negative rate of 19% and a false positive rate of 24% in diagnosing liver pathology from ultrasound scans. The results of this study support these findings. Only four patients with anomalies on the initial ultrasound scan remained abnormal three years later. Eight, including one patient with confirmed cirrhosis, were reported as normal and one child with evidence of cirrhosis on liver biopsy continues to demonstrate a "normal liver" on ultrasound.

The only way to be confident that serum GST B_1 activities accurately reflect the clinical state of the liver would be to perform an open biopsy, as even a closed needle biopsy may miss affected areas of focal fibrosis or cirrhosis. This could not be justified but would confirm that elevated GST B_1 activities are related to the permanent cirrhotic changes in cystic fibrosis rather than to potentially reversible steatosis. Serum GST B_1 activities have, however, been correlated with biopsy proven cirrhosis in adults with other diseases.[11] It is therefore necessary to draw inferences from liver function tests, clinical examination and ultrasound scans in relation to serum GST B_1. The need for non-invasive tests to predict and monitor the extent of liver disease in cystic fibrosis is likely to become more pressing as treatment alternatives such as organ transplantation or therapy with biliary conjugates develop. UDCA, by provoking a bicarbonate rich choleresis, has an established role in gallstone dissolution. More recently, it has been shown to reduce bilirubin and the hypertransaminasaemia associated with cholestatic liver disease in cystic fibrosis.[22 23] While the long term effect of UDCA on histology and prognosis is uncertain, serum GST B_1 appears from our results to have greater potential for predicting and monitoring liver disease in patients with cystic fibrosis than conventional serological or clinical tests of liver function.

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